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SYSTEMATIC ANALYSIS OF EXCEPTIONALLY PRESERVED FOSSILS: CORRELATED PATTERNS OF DECAY AND PRESERVATION

by SARAH E. GABBOTT[1,*], ROBERT S. SANSOM[2,*] *and* MARK A. PURNELL[1]

[1]School of Geography, Geology & Environment, University of Leicester, Leicester, LE1 7RH, UK; sg21@le.ac.uk
[2]Department of Earth & Environmental Sciences, University of Manchester, Manchester, M13 9PT, UK; robert.sansom@manchester.ac.uk

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Abstract: The fossil record of non-biomineralized animals and tissues provides important insight into deep-time evolutionary events. Interpretation of these highly variable remains requires an understanding of how both decay and preservation lead to fossilization. Here we establish a quantitative approach that unites data from decay experiments of extant taxa with preservation mode of fossils, allowing evaluation of both information loss and information retention, and their interaction, in non-biomineralized fossils. We illustrate our approach using fossil data from two Lagerstätten with distinct taphonomic regimes, one characterized by phosphatization, and the other by pyritization of non-biomineralized tissues. This demonstrates that frequency of occurrence of characters in fossil taxa is significantly correlated with sequences of character decay observed in extant comparator organisms, and that decay prone and decay resistant characters have distinct preservation modes; the former are mineralized and the latter are organically preserved. The methods and principles applied here to non-biomineralized vertebrates are applicable to other exceptionally-preserved fossils and allow for identification of systematic biases in fossil specimen completeness, character retention and the mode of their preservation. Furthermore, our analyses validates experimental decay in supporting the interpretation of anatomy in non-biomineralized fossils.

Key words: taphonomy, decay, mineralization, soft tissues, exceptional preservation.

EXCEPTIONALLY preserved fossils retain evidence of non-biomineralized characters and tissues, and thus provide unique windows into the deep history of life, allowing us to reconstruct evolutionary events and ancient ecosystems that would otherwise remain unknown. Correct interpretation of such fossils requires an understanding of how they were formed. By definition, any fossil is incomplete compared to the organism it once was, and even the best examples of exceptional preservation cannot be read as though they are anatomically intact because a range of post-mortem processes determine the outcome of fossilization. Which characters are lost? Which are retained? How have characters been transformed by the processes that have preserved them? (We use 'character' throughout the paper as a shorthand to refer to any aspect of anatomy.) If these questions go unanswered we risk significantly biasing all the phylogenetic, evolutionary and palaeoecological analyses that follow from anatomical interpretation of fossil remains (Donoghue & Purnell 2009; Sansom 2015; Purnell *et al.* 2018). This is particularly important for those fossils that preserve the remains of organisms that lacked any biomineralized skeletons, which includes the stem lineages of many extant phyla (Murdock & Donoghue 2011).

Fossilization reflects the interplay of processes that promote the loss of anatomical information and processes that promote the retention of information. Understanding the interactions between these processes and their impact on the nature of fossils and the fossil record is the field of taphonomy. Loss of anatomical information principally occurs through decay and decomposition. Processes that control retention of anatomical information include those through which the remains of non-biomineralized tissues are replicated by minerals, or converted into compounds which are stable over geological timescales. These processes can be broadly divided into mineralization and organic maturation. Mineralization involves replacement or templating of non-biomineralized tissues by minerals, most commonly apatite and pyrite (e.g. phosphatization (Sagemann *et al.* 1999; Butterfield 2002, 2003); pyritization (Farrell *et al.* 2009; Cai *et al.* 2012)). Such authigenic

*Authors are alphabetical.

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mineralization involves *in situ* growth of minerals and is purported to occur 'rapidly' relying on steep chemical gradients generated by the microbes which are decaying the tissues (Allison 1988; Sagemann *et al.* 1999; Butterfield 2002; Briggs 2003). Conversely, in maturation, *in situ* polymerization of more recalcitrant organic tissues occurs, resulting in geological stability of carbonaceous remains (e.g. Briggs 1999; Gupta *et al.* 2006, 2009; McNamara *et al.* 2016) although see McNamara *et al.* 2006 for an example in more labile tissues), and this is considered to occur over time during diagenesis (e.g. Gupta *et al.* 2009; Cody *et al.* 2011). For both decay and preservation the timeframe of operation, early after death or a long time after death, is important because different processes operate at different times during fossilization. In addition, rates of operation (rapid and slow) are also critical in determining which features of a carcass become fossilized and by what mode. However, much of the literature is not explicit about whether such processes are 'early or late' and/or 'rapid or slow'. This is important: rates and timing are distinct, and if different modes of preservation operate at different times after death they will have a different impact on characters that differ in their rate of decay. For example, it's possible that maturation could proceed relatively rapidly albeit acting on tissues that are slow to decay and which persist into the latest stages of character loss; maturation at this point cannot preserve more decay-prone characters because they are already lost.

Recent years have seen much progress in using experimental methods to investigate processes of decay, and in the application of new analytical approaches to determine the final composition of the organic compounds and minerals that constitute fossils. Despite huge strides in both these areas, integrating our understanding of decay processes with better data on fossil composition to decode the filters that skew the anatomical information contained in exceptionally preserved fossils has lagged behind. This is in part because taphonomic experiments have been designed to investigate processes of decay, mineralization and maturation separately, to reduce the number of variables involved (Purnell *et al.* 2018). Consequently, anatomical interpretations of fossils informed by data from decay experiments commonly include the caveat that preservation, and different modes of preservation, may act at different points in the decay trajectory (Sansom *et al.* 2011; Murdock *et al.* 2014). This gap in knowledge regarding which preservation processes act and when during decay has been part of the problem in applying decay data to fossil remains; many taphonomically informed interpretations have had to rely on qualitative narratives, and some have questioned the value of using the results of decay experiments to interpret patterns of character retention in fossils on the basis that they are applied too literally, or that this approach assumes that decay resistance alone controls which characters fossilize (Parry *et al.* 2018). Like many researchers working in this area, we subscribe to the view that qualitative comparisons of decay experiments and fossils can highlight areas of discrepancy that provide fruitful lines of further enquiry. For example, fossilization of arthropod neural tissues (e.g. Ma *et al.* 2012; Tanaka *et al.* 2013) appeared to conflict with experimental evidence that they are highly decay-prone relative to other tissues (Murdock *et al.* 2014; Sansom 2016). Noting this, Murdock *et al.* (2014) outlined four possible explanations, including the hypotheses that preservation by iron minerals occurred earlier, relative to decay, than in other Lagerstätten, and/or that nervous system tissues were preferentially preserved soon after death by an unknown mechanism; the latter explanation was also raised as a possibility by Sansom (2016). This scenario is supported by recent work by Saleh *et al.* (2020) presenting a new hypothesis of rapid fossilization via biogenic iron. This all speaks to the larger issue: in order for decay data to be used more directly to aid fossil interpretation we need to improve our ability to integrate different lines of evidence to produce more robust interpretations of exceptionally preserved fossils.

Here we articulate and test fundamental hypotheses related to the fossilization of non-biomineralized taxa and use them to develop a new quantitative framework for systematic analysis of the character composition and anatomical completeness of exceptionally preserved fossils based on statistical testing of predictive models derived from experimental data. The models that result from application of this framework will not necessarily explain all exceptionally preserved fossils, but they provide the basis upon which to understand deviations from expectations and evaluate their significance for how we read the fossil record. Our approach is underpinned by a simple hypothesis testing framework, and predictions that follow therefrom (Fig. 1). The key hypothesis we test is that, in terms of the characters present (anatomical completeness and mode of preservation of characters), variation between individuals in exceptionally preserved fossil taxa reflects the interaction between systematic patterns of character loss (principally decay) and preservational events (mineralization and maturation). These events had the potential to affect all specimens in a Lagerstätte. It follows from this that, for specimens of a fossil taxon: (1) the frequency of occurrence of characters might be expected to exhibit a relationship with their propensity to decay in extant relatives; (2) characters that are more prone to decay should be fossilized through modes of preservation (mineralization) that operate earlier in the post-mortem history of a specimen; whereas (3) characters that are more decay resistant should be fossilized through modes of preservation (including maturation) that operate later. We use early and late here in a relative,

not an absolute sense. Statistical testing of these hypotheses and predictions requires that we are able to reject the associated null hypothesis, even though some of the predictions that logically follow from this represent scenarios that are beyond what might be considered to be realistic end members in a spectrum of possible alternative taphonomic interpretations. This null hypothesis is outlined in Figure 1A.

Our hypothesis draws on the reasoning of previous work (Briggs 2003; Butterfield 2003; Purnell *et al.* 2018) that character loss through decay generates a unidirectional change in the substrate on which processes of mineralization and maturation subsequently operate (i.e. decay leads to loss of characters resulting in an irreversible decline in the anatomical completeness of a carcass). Furthermore, experimental analyses of a range of animals have demonstrated that post-mortem decay is non-random but instead goes through a series of stages (Briggs 1995), exhibiting consistent (and repeatable) sequences of character loss (Sansom *et al.* 2010*a*; Sansom *et al.* 2011; Murdock *et al.* 2014). In cephalochordates and non-biomineralized basal vertebrates, for example, certain anatomical characters are lost early during decay, whilst others consistently survive for much longer (see e.g. Sansom *et al.* 2010*a*, 2011). It also follows that if the null hypothesis is rejected and our hypothesis is correct, where exceptionally preserved taxa exhibit a range of completeness, decay-prone characters should be more common in the most complete specimens, but should tend to be absent from those that are less complete. The latter should exhibit a tendency towards preserving more decay resistant characters.

Here we use non-biomineralized vertebrates to explore the relationship between the frequency with which characters are preserved across specimens of the same taxon, and the relationship between specimen completeness and preservation mode of characters. Experimental decay data is available for lampreys, for which we can make like-for-like anatomical comparison between decay data and fossil completeness and character frequency of occurrence in exceptionally preserved lampreys (e.g. *Mayomyzon*) and closely-related fossils (e.g. *Euphanerops*). This enables quantitative exploration of the relationship between experimental anatomical decay data and the occurrence of characters in fossils and their preservation mode.

MATERIAL AND METHOD

Material

Specimens of the fossils *Euphanerops* (Woodward 1900) were studied from the collections of Musée d'Histoire Naturelle, Miguasha (MHNM) and Natural History Museum, London (NHMUK). *Euphanerops* is a vertebrate from the Upper Devonian Lagerstätte of Miguasha, Quebec (Janvier & Arsenault 2002; Janvier & Arsenault 2007; Janvier 2008; Sansom *et al.* 2013). Similar to other dominantly or completely non-biomineralized fossils, *Euphanerops* displays a range of preservation fidelity from specimens preserving many characters (or body parts) to those with fewer characters preserved (Appendix S1, Fig. S1.1).

Specimens of the fossils *Mayomyzon* (Bardack & Zangerl 1968) and *Pipiscius* (Bardack & Richardson 1977) were studied from collections of the Field Museum, Chicago (FMNH) and the Royal Ontario Museum, Toronto (ROM). Both are from the Carboniferous Mazon Creek fauna (Illinois) and have been interpreted as fossil lampreys. Some specimens having features characteristic of *Mayomyzon* (e.g. ROM V56800) also possess concentric circular mouthparts (Gabbott *et al.* 2016). This feature is characteristic of *Pipiscius* Bardack & Richardson, 1977, another jawless vertebrate from Pit 11, Essex fauna of the Mazon Creek. Given that these mouth-parts are the defining features of *Pipiscius*, and that *Pipiscius* has few other defining features (Bardack & Richardson 1977), we interpret *Mayomyzon* and *Pipiscius* as synonymous (*Mayomyzon* Bardack & Zangerl, 1968 takes priority), and we treat the specimens as a single taxon in our analyses (see Appendix S1, Fig. S1.3).

Method

Here we develop a systematic and repeatable approach to analysis of non-biomineralized fossils which allows us to explore and independently test the relationship between the characters that are preserved in a fossil, their mode of preservation, and the decay profile across specimens and characters. The relative frequency with which characters are preserved in the fossil taxon could represent random incompleteness or a range of interacting factors occurring through their taphonomic history. If, however, the fossil character frequency of occurrence correlates with the sequence of character loss in an extant comparator organism, this allows the ranking of anatomical characters in fossils to be interpreted in terms of those that are lost early and those that are progressively lost later in decay. Comparing this with data regarding preservation modes (determined from characteristic microtextures and geochemical composition of characters within specimens) allows systematic analysis of whether decay prone and decay resistant characters differ in their preservation mode.

Morphological characters and or body parts (for simplicity referred to as 'characters' throughout) for each fossil taxon were observed in every specimen using a

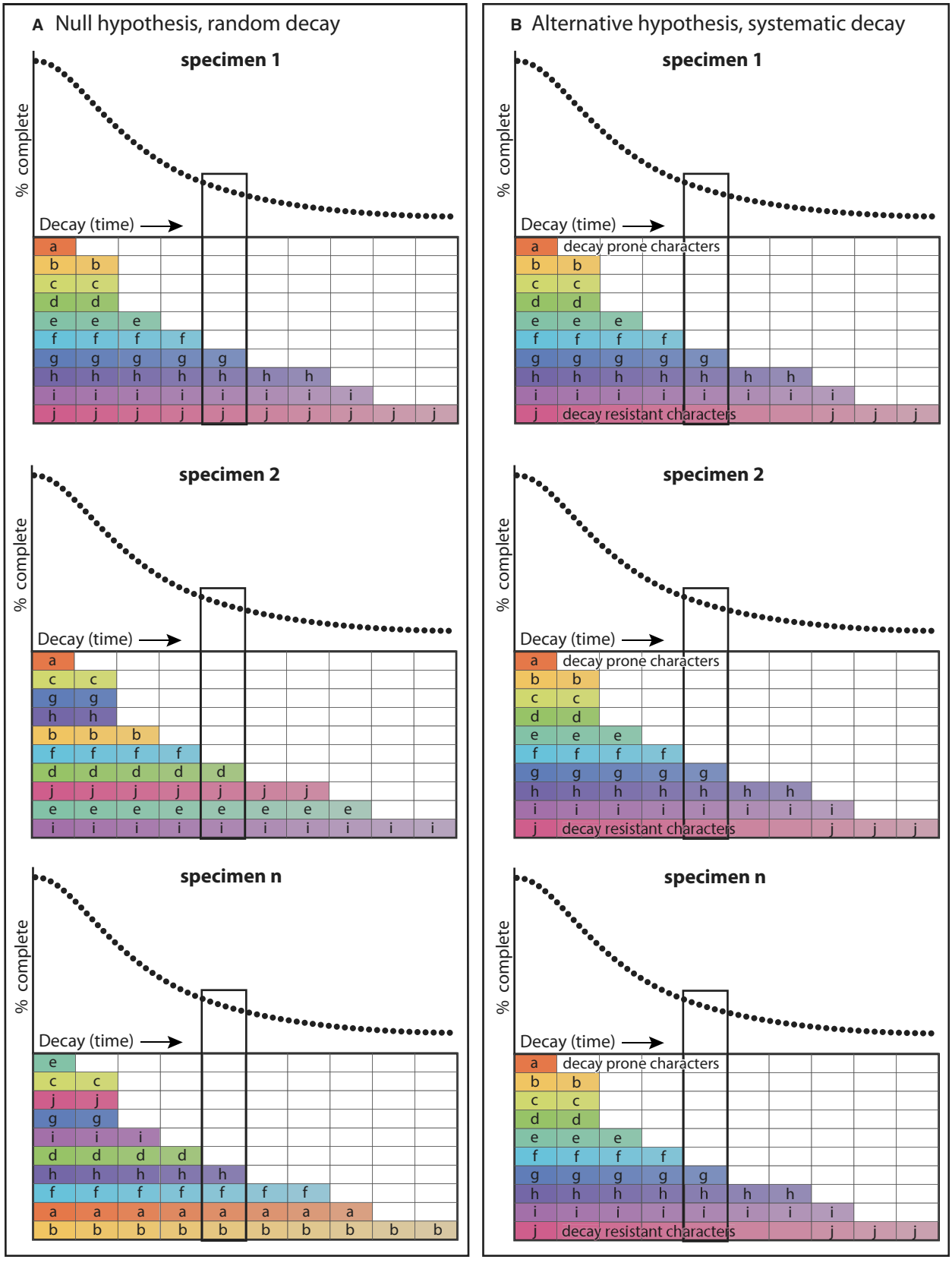
A Null hypothesis, random decay
B Alternative hypothesis, systematic decay
specimen 1
specimen 2
specimen n
% complete
Decay (time)
decay prone characters
decay resistant characters

FIG. 1. Simplified models of character loss through decay to illustrate the null hypothesis and the alternative. Characters are represented by letters in coloured boxes. Columns can be read either as sequential steps in the decay of an individual, or as a series of specimens exhibiting increasing amounts of decay. A, according to the null hypothesis, the sequence of character loss is not-systematic, and differs between specimens; specimens in which decay is halted (or fossilization occurs) at the point in time represented by the box will differ in what characters are present, even though the amount of decay and character loss is the same. B, in the alternative hypothesis, which is supported by the results of decay experiments, character loss is conserved between specimens (and can be conserved across taxa); specimens in which decay is halted (or fossilization occurs) at the point in time represented by the box will not differ in what characters are present.

combination of photography (variously with low angle light, polarized light and filters, and submersion in alcohol or water), camera lucida drawings, and optical binocular microscopy. Characters were scored as present (1) or absent (0) (Figs 2, 3). In the cases where the topological region bearing a morphological character is missing or obscured (e.g. part of the specimen is missing), it was scored as equivocal (–). The presence of characters/body parts was described and analysed independently of any interpretation of anatomical homology (see Sansom *et al.* 2010*b* for discussion). Compositional data were also collected for each observed character in each specimen where possible (Figs 2, 3). Specimens were analysed using an environmental scanning electron microscope with energy-dispersive x-ray spectroscopy to collect composition data and microtextural data (Hitachi S-3600N SEM with Oxford INCA 350 EDX, Zeiss Sigma 300 and FEI Quanta 650 in partial vacuum at 5–15 kV). Alongside composition, microtexture can be used to discriminate carbonaceous anatomy as being pigmented or non-pigmented; the former comprising elliptical to oblate microbodies shown to be melanosomes (Gabbott *et al.* 2016). Direct analysis of all characters in all specimens was not possible (e.g. if specimens are on slabs of rock too large for an SEM chamber to accommodate), so in some instances composition was inferred through comparison with other directly observed characters in other specimens with similar textures, colour and appearance (Figs 2, 3).

The presence–absence matrix of characters in the fossil specimens was translated into a spectrum of specimen completeness by ranking specimens from most complete (those with the highest tally of characters) to the least complete (those with the lowest tally of characters). Similarly, the variation in frequency of occurrence of fossil characters across all specimens of the same taxon was translated into a spectrum by ranking characters according to relative frequency. In this form, the least frequently preserved characters, present only in the most complete specimens occur towards the top left of the matrix. The most frequently preserved characters preserved in the least complete specimens are towards to bottom right (Figs 2, 3).

Ranking a presence–absence matrix in this way has the potential to produce an apparent pattern in random data; consequently we tested the null hypothesis that variation in character frequency and specimen completeness is random by comparing the observed fossil spectra with ranked distributions resulting from random reallocation of characters to specimens, and randomization of specimen completeness. This was achieved by taking character entries (in rows) and randomly reallocating them to columns (equivalent to specimens in the original matrix). The frequency of occurrence of each character is unchanged, but specimen completeness is allowed to vary and is unrelated to which characters are present; the process produces a simulated set of random 'specimen completeness' data. Randomization of character frequency was achieved by randomly reallocating character entries within specimens (in columns). Specimen completeness is unchanged, but which characters are present in a specimen is allowed to vary and is random (i.e. for a given level of completeness all characters have an equal probability of being present); the process produces a simulated set of random 'character frequency' data. Each process was repeated 100 times, and the simulated specimen completeness and character frequency data compared with the observed fossil spectra. The null hypothesis is rejected if the observed distributions of character frequency and specimen completeness fall outside the range of randomized distributions. We were able to reject the null hypothesis that variation in character frequency and specimen completeness is random (i.e. that the spectra for *Euphanerops* and *Mayomyzon* are simply an artefact of systematically sorting random data; see Appendix S1, Fig. S2.1). Rejection of this null hypothesis allowed us to test the hypothesis that patterns of character frequency and specimen completeness are taphonomically controlled. This involved first testing whether the frequency of occurrence of characters in the fossils is correlated with the decay resistance of homologous characters as observed in experimental decay (*Lampetra*; see Sansom *et al.* 2011). Second, we tested the hypothesis that patterns of character frequency and specimen completeness are linked to the mode of their preservation (see below for details).

To test the hypothesis that the frequency of occurrence of characters in the fossils is correlated with their decay resistance as observed in experimental decay of extant comparator taxa characters were homologized following

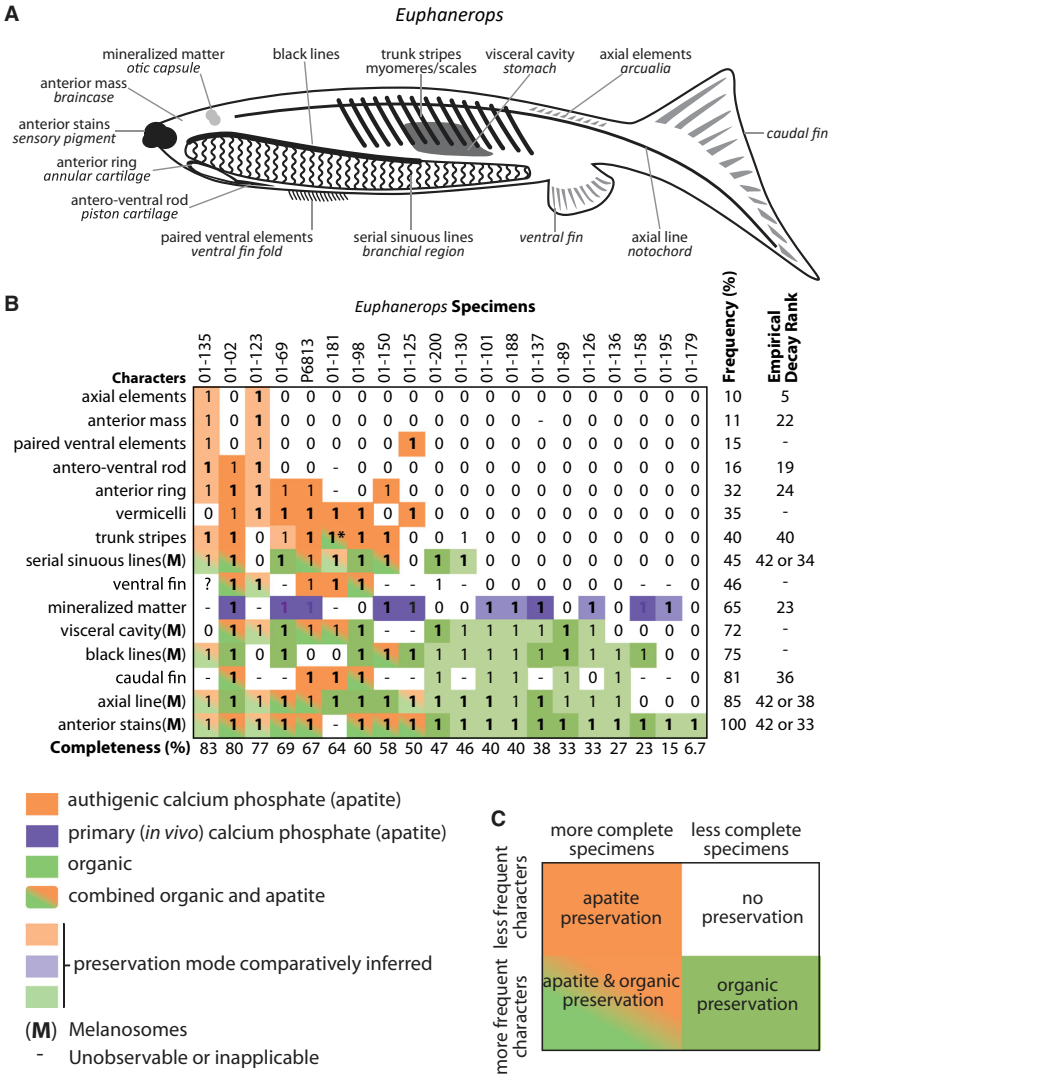

FIG. 2. *Euphanerops* anatomy and preservation modes. A, anatomy of *Euphanerops* with characters and their interpreted homology (*italics*). B, table of character presence and absence across specimens (columns) and the frequency of occurrence of characters (rows), with observed and inferred preservation modes indicated for each morphological character by the cell colour; both specimen completeness and character frequency are ranked: specimen completeness by ranking specimens from most complete (those with the highest tally of characters) to the least complete (those with the lowest tally of characters) and frequency of occurrence of fossil characters by ranking from the least frequently occurring characters across all specimens to the most frequently occurring. C, simplified schematic of the table in B showing the general distribution of specimen completeness and frequency of characters with relationship to mode of preservation; the more complete specimens of *Euphanerops* have morphological characters largely composed of apatite and carbon, whilst the less complete specimens have characters composed primarily of carbon; similarly, the characters that occur the least frequently across all specimens are comprised of apatite whilst the more frequent and widely distributed characters are composed of either carbon alone or carbon and apatite together.

the approach advocated by, for example, Sansom *et al.* (2010*b*) and Donoghue & Purnell (2009). The most appropriate extant comparator is the lamprey, and we used the adult *Lampetra* decay sequence (Sansom *et al.* 2011). Because some fossil characters exhibit organic preservation with textures indicating they are pigment, we used a decay sequence for *Lampetra* that takes pigmentation of characters into account. For example, the eyes in *Mayomyzon* are evident as pigmented 'eye spots' representing the retinal pigmented epithelium (Gabbott *et al.* 2016) not the other tissues of the eye. Some fossil characters could not be confidently homologized with lamprey characters for which decay data is available (Sansom *et al.* 2011). For example, the 'vermicelli' and 'black

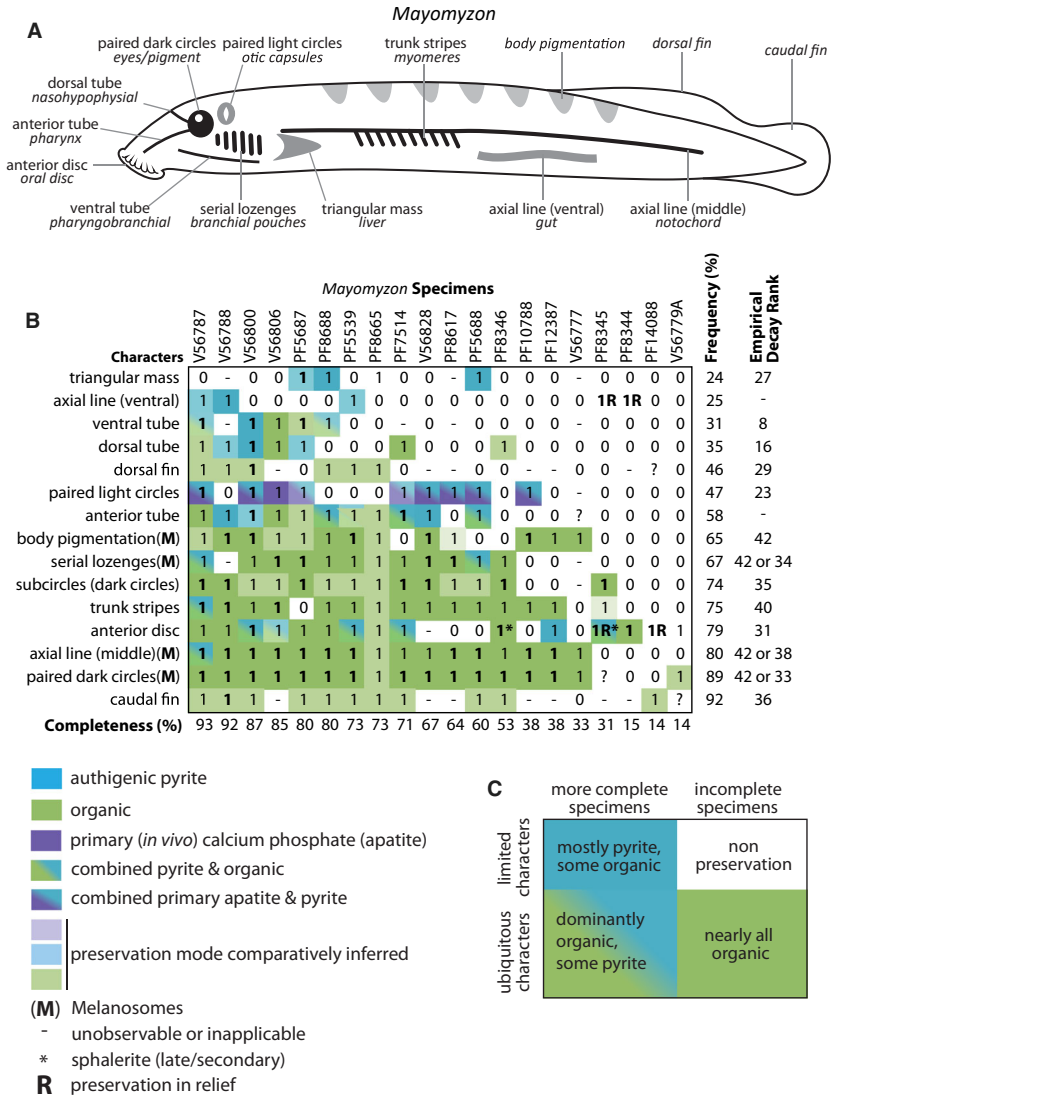

FIG. 3. *Mayomyzon* anatomy and preservation modes. A, anatomy of *Mayomyzon* with characters and their interpreted homology (italics). B, table of character presence and absence across specimens (columns) and the frequency of occurrence of characters (rows), with observed and inferred preservation modes indicated for each morphological character by the cell colour; both specimen completeness and character frequency are ranked: specimen completeness by ranking specimens from most complete (those with the highest tally of characters) to the least complete (those with the lowest tally of characters) and frequency of occurrence of fossil characters by ranking from the least frequently occurring characters across all specimens to the most frequently occurring. C, simplified schematic of the table above showing the general distribution of specimen completeness and frequency of characters with relationship to mode of preservation; the more complete specimens of *Mayomyzon* have morphological characters largely composed of pyrite and organic carbon, whilst the less complete specimens have characters composed primarily of organic carbon; similarly, the characters that occur the least frequently across all specimens are comprised of pyrite whilst the more frequent and widely distributed characters are composed of either organic carbon alone or carbon and pyrite together. Rare ZnS occurs but is not confined to anatomical features, it also occurs in the sediment near the specimens and is likely to be very late and secondary in origin.

lines' (Janvier & Arsenault 2007) of *Euphanerops* have no clear homologues in extant lamprey. Furthermore, decay data are not available for stomach, gut, anal fin or pharynx. Correlation between frequency of occurrence of fossil characters and decay sequence of characters in *Lampetra* was calculated using Spearman's rank correlation.

The relationship between patterns of character frequency, specimen completeness, and the mode of preservation of characters was investigated using Spearman's rank correlation and *t*-tests. We tested two specific hypotheses: that the frequency of occurrence of characters is correlated with the frequency with which they are

preserved in a particular mode (Spearman's rank); and that characters preserved in different modes differ in their relative propensity to decay (i.e. decay prone characters are preserved in modes we would expect to act relatively early with respect to decay, and decay resistant characters are preserved in modes we would expect to act relatively late). Preservation in *Euphanerops* was scored according to the frequency with which a character is preserved in apatite or as organic remains. Some characters exhibit both modes of preservation, so the categories are not mutually exclusive (e.g. a character that is present in 18 specimens and is preserved as organics in all of them, but with apatite in nine specimens would get a score of 100% for organic and 50% for apatite); *t*-tests required characters to be classified according to mode of preservation. Characters in *Euphanerops* were classified as preserved in apatite, rather than as organic, where the character exhibited apatite preservation in all specimens in which it was found (independent of the number of specimens exhibiting organic preservation of the character). In *Mayomyzon*, most characters exhibit both organic and pyrite modes, sometimes in the same specimen; characters were classified as preserved in pyrite where the character exhibited pyrite preservation in 40% or more of specimens in which it was found. For most characters in *Mayomyzon*, classification as organic equates to <10% of specimens exhibiting any pyrite preservation of the character.

RESULTS

Fossil mode of preservation

The composition of characters in all specimens was determined through SEM-EDX and textural analyses. EDX analyses of fossil characters demonstrated that they are composed of carbonaceous material, and mineral phases comprising calcium and phosphorus (apatite) and iron and sulfur (pyrite). An individual fossil may consist of a combination of preservation modes, or just one (Figs 2, 3). *Mayomyzon* possesses characters preserved as carbonaceous material, apatite and pyrite; *Euphanerops* as carbonaceous material and apatite. The carbonaceous characters generally comprise either a thin, commonly fractured film, similar to those in Burgess Shale fossils (e.g. Butterfield 1990; Page *et al.* 2008), or consist of microbodies of a size and distribution identical to melanosomes in *Mayomyzon* (Gabbott *et al.* 2016), indicating that these characters were pigmented in life (Fig. 3). Both textures can also co-occur. Characters composed of apatite show textures ranging from smooth and consolidated to porous and fractured (Fig. 4; Fig. S1.2 is in the Appendix S1). The paired 'mineralized masses' in *Euphanerops* (*sensu* Janvier & Arsenault 2007) located in the anterior region comprise numerous spheres composed of apatite which are extremely smooth (diameter 5–15 μm; Fig. 4E); they occur in the otic region (Janvier & Arsenault 2007) and are likely to be statoliths. In *Mayomyzon*, similar smooth apatite spheres occur in the centre of pyrite rings behind the eyes and have been interpreted as the statoconia within an otic capsule by Gabbott *et al.* 2016 (diameter 5–15 μm; Fig. 4). For both taxa, because our focus is on decay and preservation of non-biomineralized characters, the otic capsules were excluded from statistical analyses relating to preservation, but results including the otic capsules are similar and do not change the outcome, although test values differ (see Appendix S1). In *Mayomyzon*, characters defined by elevated Fe and S comprise minerals with a euhedral–subhedral octahedral, and, in some instances, framboidal habit which is consistent with interpretation as pyrite (Gabbott *et al.* 2016). In addition, in *Mayomyzon* there are rare instances of sphalerite (ZnS) occurring within the fossil margin but not restricted to any anatomical feature, and some characters, most notably the oral disc, are preserved primarily in relief (Gabbott *et al.* 2016).

Correlated patterns of decay and preservation: Euphanerops

In terms of the characters present in the fossils, the most complete specimen is only 83% complete, with other specimens exhibiting a continuous decline in completeness to specimens that have fewer than 10% of the characters (Fig. 2). The frequency of occurrence of characters is significantly correlated with their resistance to decay in *Lampetra* ($R_s = 0.898$, $p = 0.001$), that is, the more decay prone characters are rarely exhibited in fossils, whilst the more decay resistant characters occur frequently in fossils. Correlations with mode of preservation are also strong: the frequency of occurrence of characters is correlated with the relative frequency with which they are preserved in apatite (strong negative correlation; $R_s = -0.840$, $p = 0.0002$) and the relative frequency with which they are preserved as organic remains (strong positive correlation; $R_s = 0.895$, $p < 0.0001$). Similarly, mode of preservation (relative frequency with which characters are preserved in apatite/organic) is correlated strongly, negatively and positively, with their resistance to decay in *Lampetra* ($R_s = -0.780$, $p = 0.013$; $R_s = 0.938$, $p = 0.0002$, respectively). Comparing the propensity to decay of characters that in *Euphanerops* are preserved in apatite with those preserved as organic remains yields a similar result. Characters that are preserved as apatite are less decay resistant ($t = -2.855$, d.f. 7, $p = 0.012$), and are preserved less frequently ($t = -6.106$, d.f. 12, $p < 0.0001$). These results allow us to unequivocally reject the null hypotheses regarding fossilization: the frequency of occurrence of characters in *Euphanerops* is correlated with their

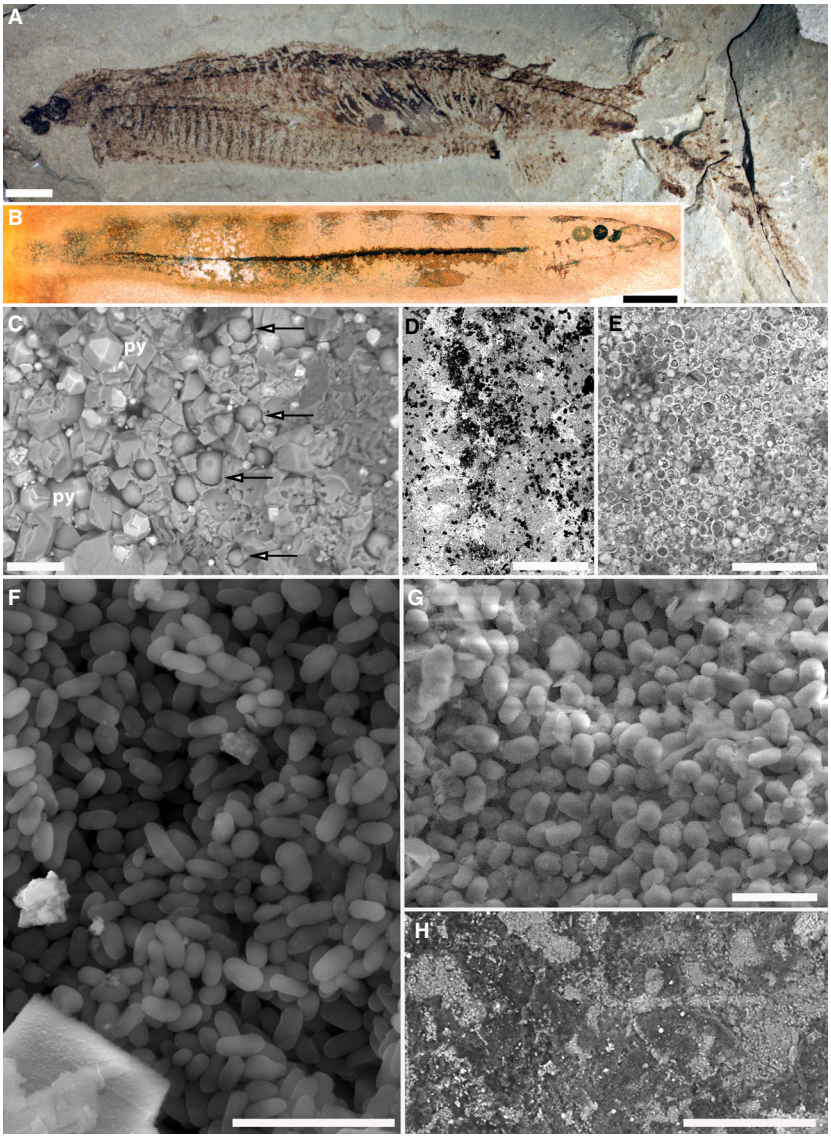

FIG. 4. *Euphanerops longaevus* (Escuminac Formation, Miguasha, Quebec, Canada: MHNM 01-02a) and *Mayomyzon pieckoensis* (Mazon Creek, Illinois, USA: ROMV5800b). A, complete *Euphanerops* (see Fig. 2 for labelled anatomy). B, complete *Mayomyzon* (see Fig. 3 for labelled anatomy). C–H, scanning electron microscopy back scatter electron (SEM BSE) images. C–E, mineralized anatomy in *Mayomyzon* (C) and *Euphanerops* (D, E): C, statoliths (arrows), biomineralized as calcium phosphate *in vivo*, and typical euhedral pyrite crystals (py), which mineralize authigenically post mortem; D, 'vermicelli', light loop-like structures which are 'phosphatized' post mortem, and black organic carbon; E, mass of spherical structures composed of calcium phosphate which we interpret as biomineralized statoliths. F–H, organically-preserved anatomy in *Mayomyzon* (F) and *Euphanerops* (G, H): F, melanosomes in the branchial area; G, melanosomes in the 'anterior stain'; H, organic carbon film with cracked appearance in the 'anterior stain'. Scale bars represent: 10 mm (A); 5 mm (B); 15 μm (C); 500 μm (D); 25 μm (E); 2.5 μm (F); 2 μm (G); 125 μm (H).

resistance to decay, and characters preserved in apatite are more decay prone, and present in fewer specimens, than those preserved as organic remains.

Finally, analysis of specimen completeness (number of characters) shows a strong correlation with the proportion of characters preserved in apatite ($R_s = 0.823$, $p < 0.0001$). Specimens with apatite preservation of characters, which character-based analysis shows are the less decay resistant characters, are more complete.

Correlated patterns of decay and preservation: Mayomyzon

In terms of characters present in the fossils, the most complete *Mayomyzon* specimen is 93% complete, with a decline to two specimens with 14% of the characters (Fig. 3). The frequency of occurrence of characters across specimens is significantly correlated with their resistance to decay in *Lampetra* ($R_s = 0.648$, $p = 0.023$). The correlations with mode of preservation are not as strong as those seen in *Euphanerops*. Three characters in four specimens have an unknown mode of preservation, so tests were carried out both including and excluding these to assess the degree to which the results are affected. The outcomes remain the same (although test values differ). The frequency of occurrence of characters is correlated negatively with the relative frequency with which they are preserved in pyrite ($R_s = -0.671$ $p = 0.0087$ and $R_s = -0.7423$ $p = 0.0023$, respectively including and excluding characters with unknown mode of preservation).

The relative frequency with which characters are preserved in pyrite is not correlated with their resistance to decay in *Lampetra* ($R_s = -0.576$, $p = 0.0501$ and $R_s = -0.0554$ $p = 0.0619$, respectively including and excluding the four specimens where three characters have unknown mode of preservation). To explore these patterns further we compared characters preserved in pyrite with those preserved as organic remains: they differ significantly in terms of decay rank and in terms of frequency of occurrence of characters. (Note that we used a two tailed test because the hypothesis being tested is that there is a difference, not that pyrite preserves decay prone characters.) These tests reveal that characters that are frequently preserved as pyrite are less decay resistant ($t = -4.203$, d.f. 10, $p = 0.0018$), and are preserved less frequently ($t = -3.234$, d.f. 12, $p = 0.0072$ and $t = -3.814$, d.f. 12, $p = 0.0025$, respectively including and excluding the four specimens where three characters have unknown mode of preservation) than characters that are rarely preserved in pyrite (<20% of specimens have pyrite preservation). This provides broad support for the hypothesis that pyrite preservation is more common for characters that are less decay resistant (and less frequently preserved), but the lack of a significant correlation between frequency of pyrite preservation and decay resistance in extant *Lampetra* indicates that the relationship is not as strong as it is in *Euphanerops*. That the five characters preserved exclusively as organic remains (no pyrite) are the most decay resistant characters adds weight to this relationship: pyritization occurs in less decay resistant characters.

In *Mayomyzon*, analysis of specimen completeness (number of characters) shows a strong correlation with the proportion of characters that are preserved in pyrite ($R_s = 0.560$, $p = 0.0102$ and $R_s = 0.575$, $p = 0.0079$, respectively including and excluding the four specimens where three characters have unknown mode of preservation). Specimens with pyrite preservation of characters, which character-based analysis demonstrates tend to be the less decay resistant characters, are more complete.

DISCUSSION

For the first time, we are able to demonstrate through quantitative statistical analysis that the frequency of character occurrence in non-biomineralized fossil specimens reflects the sequence in which characters decay in a carcass: fossil characters that are preserved in fewer specimens are the characters observed to be the most decay prone in extant comparator organisms, whilst the fossil characters that preserve more frequently were the most decay resistant characters. Establishing this allows us to constrain how different preservation pathways may have operated during the decay profile of the characters within a carcass; effectively we are able to test whether decay prone and decay resistant characters differ in their mode of preservation. In *Euphanerops*, the two distinct modes of preservation showed a marked pattern with respect to character distribution and decay profile. The characters that occur less frequently and were decay prone exhibit an apatite composition consistent with their replacement through authigenic phosphatization (e.g. Wilby 1993), whereas the more frequently occurring characters which were decay resistant are organically preserved. Similarly, in *Mayomyzon*, pyritization is the mode of preservation associated with characters that occur less frequently and were more decay prone, whilst organic preservation associates with more frequently occurring and decay resistant characters. Both patterns are significant; character frequency of occurrence is inversely correlated with proportion exhibiting phosphatization or pyritization. The most anatomically complete specimens are those with both mineralized and organically preserved characters: multiple preservation modes capture characters across a range of decay susceptibility (Figs 2, 3, 5, 6). The observed patterns provide empirical support for the concept that more decay prone characters are captured via authigenic mineralization (phosphatization), whilst more decay resistant characters are captured as organic remains (see Briggs 2003; Butterfield 2003).

We can assume that *Euphanerops* and *Mayomyzon* originally possessed characters that are not preserved in any specimens, so we don't know how incomplete any specimen is compared with its living representative. Nevertheless, even the most complete specimens of both taxa do not possess all known preservable characters suggesting that reconstructions of exceptionally preserved fossils focused on one or two of the 'best preserved' specimens may fail to record the complete suite of characters known to be preserved. Further scrutiny of how preservation mode relates to character frequency in *Euphanerops* provides insights into how mechanisms of preservation may or may not operate and interact along the decay trajectory; Figure 5 provides a graphical summary of this. For example, 11 of the 20 specimens have no authigenic phosphatized characters and exhibit organic preservation only (Fig. 2). The absence of early authigenic mineralization may be accounted for because either: (1) potentially mineralizable characters were lost to decay before the authigenic mineralization preservation window could operate; or (2) the conditions promoting the authigenic mineralization preservation pathway did not operate at all in these specimens. In specimens where authigenic mineralization does occur, it is not necessarily limited to early decaying characters; it can also occur to some degree in decay resistant characters that also exhibit organic preservation (Fig. 2). This

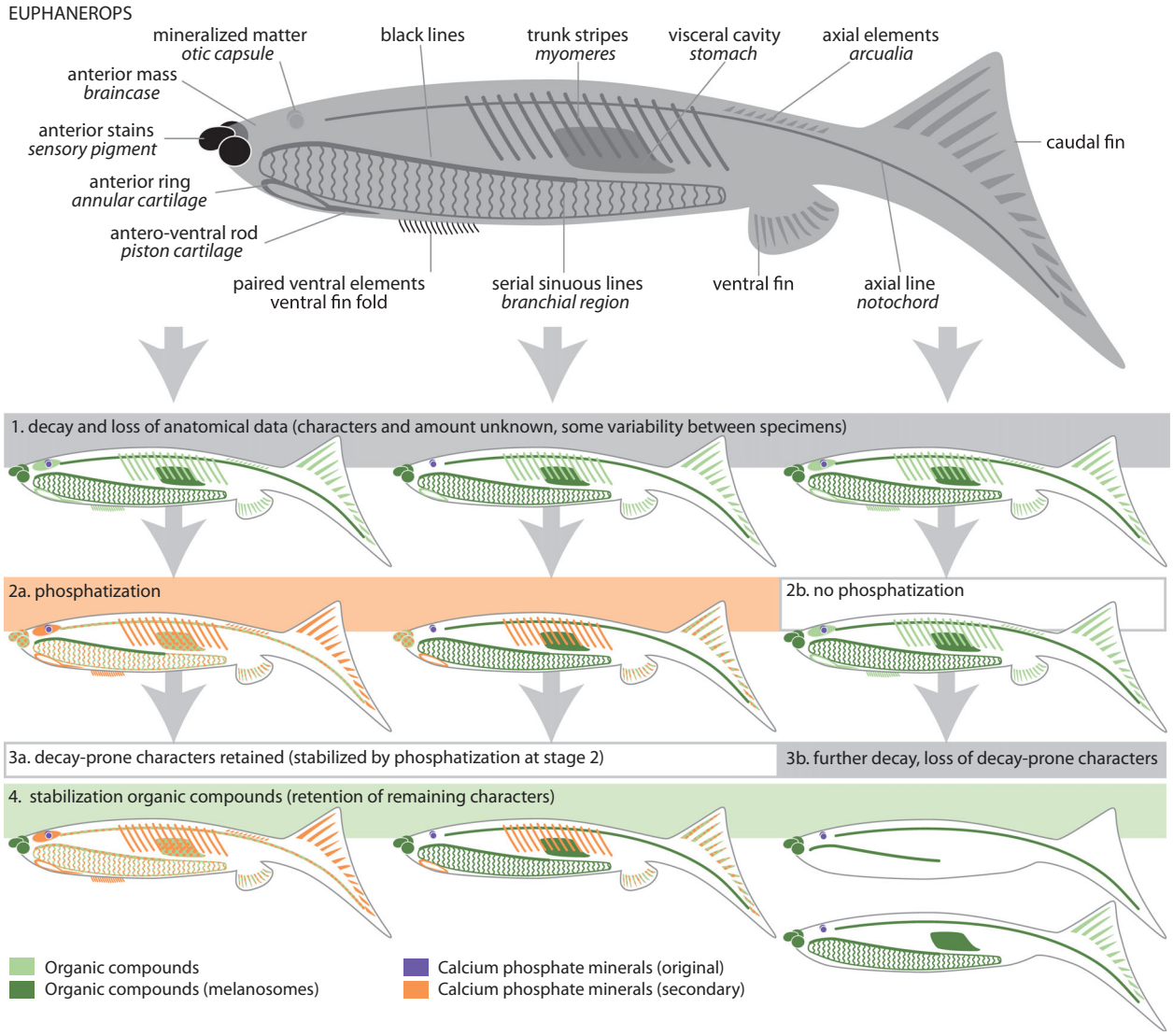

FIG. 5. Schematic illustration of how decay trajectory of different characters and preservation processes interact to produce *Euphanerops* specimens. Some fossil specimens preserve both decay prone (as calcium phosphate) and decay resistant (as organic) characters. These specimens are the anatomically most complete. Less complete specimens preserve only organic decay resistant characters because they did not experience phosphatization (2b), either because this was not operating, or because decay proceeded so rapidly that characters were lost before phosphatization could capture them. In this context it is important to bear in mind that the amount of decay experienced by a character and/or specimen is dependent on both decay rate and timing of operation of processes which replace or stabilize and preserve soft tissues (i.e. rapidly acting modes of preservation, such as phosphatization, may not necessarily act soon after death occurs).

may be explained because individual characters can comprise multiple tissue types with different decay profiles (e.g. fatty, collagenous and pigmented tissues) resulting in multiple preservation modes. For example, in many of the characters exhibiting apatite and organic composition the organic component is melanosomes (six of eight characters; Fig. 2). Here, early-decaying components of individual characters were mineralized, but in addition melanosomes record highly recalcitrant components of the same characters. Finally, the pattern of combined pathways (apatite plus organic) in some decay resistant characters may also constrain the timing of phosphatization, indicating that the loss of characters to decay prior to operation of the phosphatization window is unlikely to control the preservation of decay prone characters alone. Those specimens that preserve only decay resistant characters may do so because they were not subjected to phosphatization at any point, and

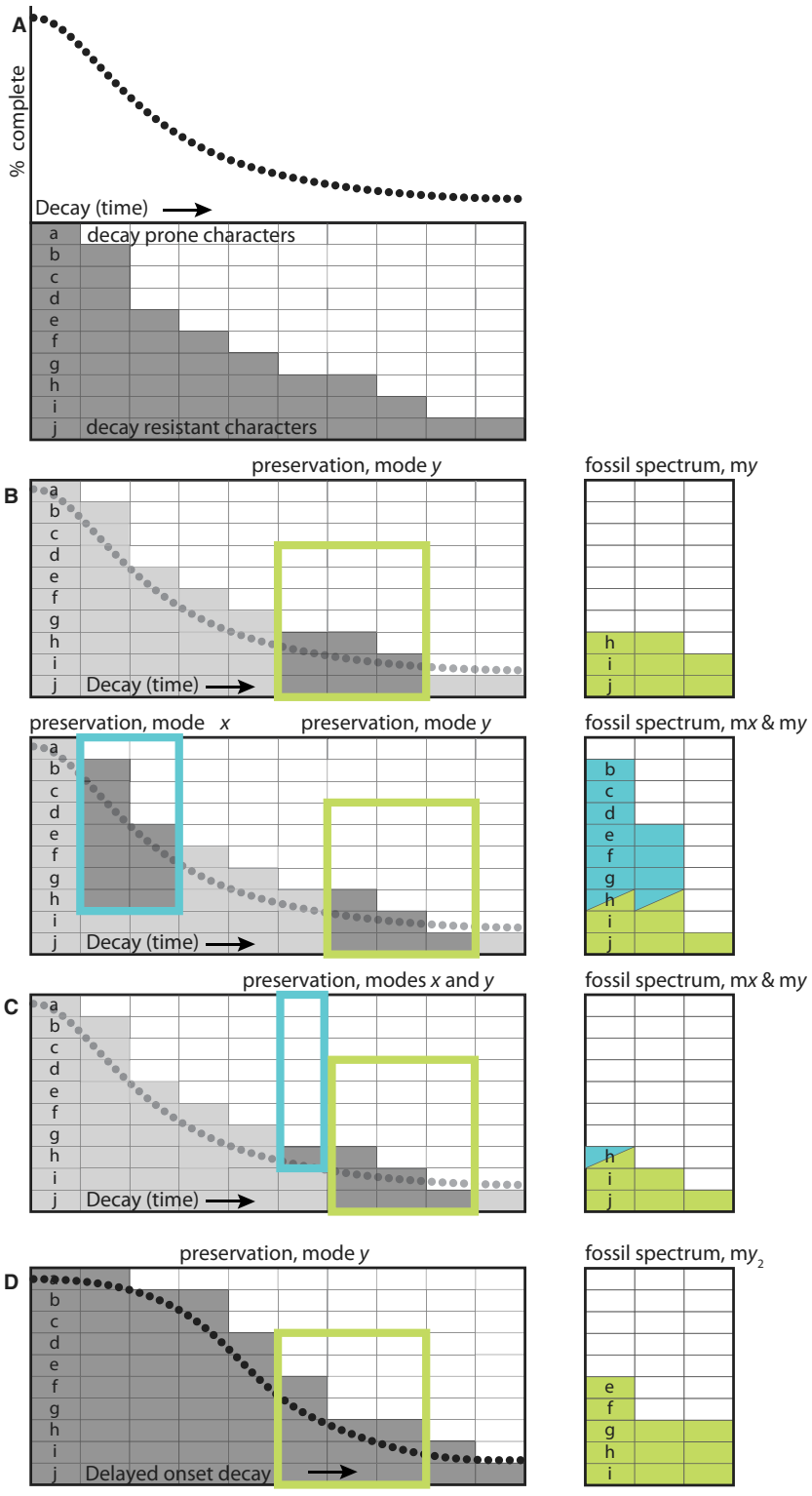

FIG. 6. Hypothetical schematic, which can be applied to any non-biomineralized fossil taxa, to illustrate the interaction of decay and preservation and how this controls which characters are preserved, the completeness of specimens and the mode of preservation of characters. A, curve represents trajectory of decay (character loss through time); the grid illustrates schematically what characters are retained: rows a–j represent different characters; columns can be read either as sequential steps in the decay of an individual, or as a series of specimens exhibiting increasing amounts of decay; in the 'fossil spectra' columns are representative of different individuals. B, the anatomical composition of non-biomineralized fossils reflects the point in the decay trajectory when preservation mechanisms operate, and the range of decay over which they operate; it is possible for some characters to exhibit more than one preservation mode (as in the lower grid with two preservation modes). C, note that the same preservation mode may not necessarily preserve the same characters if it acts at a different point in the decay trajectory. D, because what is preserved reflects the interaction of decay and preservation the timing of decay in relation to preservation is important. If the onset of decay is delayed (curve moves to the right), but preservation acts at the same time (and over the same range of decay) it has the effect of producing fossils that would seem to be the result of earlier acting preservation (preserving more characters and resulting in more complete fossils; compare with B).

decay prone characters decayed away before maturation of organic decay resistant characters (Fig. 5).

Our results demonstrate consistent and predictable patterns in character occurrence and preservation in non-biomineralized fossil vertebrates that are consistent with patterns of decay in anatomically comparable extant organisms. Our approach can be applied to other fossil taxa which show a range of 'preservation states' to

illustrate the interaction of decay and preservation and how this controls which characters are preserved, the completeness of specimens and the mode of preservation of characters (Fig. 6). It has been recognized previously that application of decay data to fossil data requires consideration and understanding of the modes of preservation in operation for the fossil taxa in question and this has been explicitly applied in many recent examples (Sansom *et al.* 2011; Murdock *et al.* 2014; Sansom 2016; Purnell *et al.* 2018). However, the preserved remains of the polychaete (*Rollinschaeta myoplena*) from the Hakel and Hjoula Lagerstätte (Late Cretaceous) were purported to represent a substantial deviation from what is normally expected from polychaetae taphonomy. In these specimens, muscles are exquisitely preserved through phosphatization but external chaetae and aciculae, known from polychaete decay experiments to be decay resistant (Briggs & Kear 1993), were poorly preserved (Wilson *et al.* 2016). This distribution of characters is consistent with the taphonomic framework proposed here. In this instance more decay prone muscles were mineralized early by microbially mediated replacement/templating by calcium phosphate, a mode of preservation that would not have affected the recalcitrant chaetae and aciculae. After the phosphatization window 'closed' (perhaps limited by phosphorus abundance) more recalcitrant tissues continued to decay because organic maturation processes were absent or limited, leading to loss or poor-preservation of recalcitrant characters. A similar taphonomic pathway was inferred to account for the apparent anomaly of the preservation of decay prone muscles (via phosphatization) as opposed to the decay resistant cuticle in the problematic fossil *Myoscolex* from the Cambrian Emu Bay Shale (Briggs & Nedin 1997).

CONCLUSION

Our approach advocates taphonomic analysis and the search for systematic patterns and biases, building on previous work to provide a statistical and modelling framework for robust quantitative analysis. The methods we describe are widely applicable to the many other important instances of soft tissue preservation. The interplay between decay and preservation mode can be conceptualized and applied to allow predictions about whether characters are taphonomically unlikely to occur, their absence reflecting non-fossilization rather than reflecting real phylogenetic absence (see Fig. 6). This distinction between phylogenetic absence and taphonomic loss is crucial for interpretations of fossil anatomy and thus fossil affinity (Donoghue & Purnell 2009; Sansom *et al.* 2010*a*; Sansom 2015). Finally, even if circumstances differ between individual fossils within a Lagerstätte, the systematic approach we propose allows deviations to be evaluated in a comparative context avoiding *ad hoc* specimen specific narratives.

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SUPPORTING INFORMATION

Additional Supporting Information can be found online (https://doi.org/10.1111/pala.12571):

Appendix S1. (1) Images of specimens and anatomical features of the fossil specimens in this study; (2) Additional statistical results not reported in the main text; (3) A schematic interpretation of how decay and preservation mode interact in *Mayomyzon*; and (4) A consideration of factors that may correlate with specimen completeness and preservation mode in *Euphanerops*.

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